

Research Article

**IMMUNO-CASTRATION BY IMMUNIZATION WITH GnRH IN
BLACK BENGAL BUCKS (*CAPRA HIRCUS*)**

A.K. Mandal^{1*}, A. Maity²

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ABSTRACT: The present study was carried out to elucidate the castration effects of immunization against GnRH (Gonadotropin releasing hormone) in Black Bengal bucks. Thirty (30) Black Bengal bucks under study were divided into three groups, viz. Group I (control animals), Group II (surgical castrates) and Group III (immunized bucks). Anti-GnRH antibody titer was measured from the blood samples collected from Group III animals on days 0, 1, 3, 5, 7, 15, 30, 45, 60, 85, 100, 115. Plasma testosterone and cortisol level were also measured for all blood samples collected (during the said period) from all the animals. The increased antibody titer in bucks about 45–50 days after primary immunization coincided with the decreasing plasma testosterone level during the same period. No significant anti-GnRH activity was observed in the control as well as surgically castrated bucks. Plasma testosterone level increased progressively during the experimental period in the control group, whereas surgical castrates showed a marked reduction in testosterone level soon after the castration. The immunized bucks were under less stress condition than the surgically castrated ones as indicated by their plasma cortisol concentration. Both the surgical and immuno-castrates were easily handled and managed during the experimental period due to a marked reduction in sexual behavioral measures than the control group. So, the immunization against GnRH may be a non-invasive, convenient alternative to the surgical castration.

Key words: Immuno-castration, GnRH immunization, Black Bengal bucks.

INTRODUCTION

The domestic male animals are commonly castrated to improve meat quality, reduce aggressive behavior and for easy management practices (Kiyma *et al.* 2000). There are a number of methods available for castration of male animals, namely surgical method (Walker and Vaughan 1980), Burdizzo or clamping techniques to crush the spermatic cords (Macaulay 1989), chemical castration (Kar *et al.* 1965, Cohen *et al.* 1990, Mitra and Samanta 2000). Such techniques of castration, however, have some demerits (primary insult and secondary consequences). Moreover, all these castration methods require additional labor, increase stress, reduce efficiency and decrease the rate of weight gain of the subjected animals. Improper

dosing of chemical agents (in case of chemical castration) may even lead to the fatality (FDA 2003).

Rising consumer awareness about the welfare of production animals, with the physical castration of livestock receiving considerable attention was emphasized in the Global Meat News' State of the Industry Survey Report (2015) as well as in the European Union's decision to voluntarily ban the physical castration of piglets without anesthesia from 2018 (Font-i-Furnols *et al.* 2012). Despite a faster growth rate and superior feed efficiency of intact males than castrates (Sales 2014), various management and welfare issues exist regarding the raising of intact males (Price *et al.* 2003). Heavy intact male carcasses

¹Department of Veterinary Anatomy and Histology, ²Department of Veterinary Biochemistry, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal & Fishery Sciences, Kolkata-700 037, West Bengal, India.

*Corresponding author. e-mail: arunmandal_2005@yahoo.com

also suffer from various meat quality issues at the abattoir. Thus, boars over 100 kg (approximately 22 weeks old) and bulls or rams with one or more permanent incisors receive a lower price tag. There is thus a need to formulate alternative management practices to ensure efficient growth of male animals resulting in optimum carcass and meat quality, without compromising animal welfare (Needham *et al.* 2017). Accordingly, the alternative methods of castration aimed to alleviate stress and discomfort as compared to the conventional procedures are of great interest. Techniques have been developed to achieve many of the effects of castration by inducing immuno-neutralization of hormones of the hypothalamic-pituitary-testicular axis (Adams and Adams 1992, Finnerty *et al.* 1996). Active immunization against GnRH, commonly known as immuno-castration, has been proposed as an alternative to traditional methods in bulls (Robertson *et al.* 1979). It is relatively non-invasive and less stressful procedure. Such immunization against GnRH is reported to suppress testicular growth as well as secretory activity in ram lambs (Schanbacher 1982, Cui *et al.* 2003, Ulker *et al.* 2005). Moreover, immuno-neutralization of GnRH inhibits spermatogenesis and decreases reproductive and aggressive behavior in domestic animals (Awoniyi *et al.* 1988, Jago *et al.* 1997, Rydhmer *et al.* 2010). Immuno-castration of pigs with a gonadotrophin releasing factor (GnRF) vaccine 'Improvac TM' (Pfizer Ltd) significantly reduces the occurrence of unwanted aggressive and sexual behaviors compared with unvaccinated control male pigs (Brewster and Nevel 2013). Much works were done in this regard in bull, stallion, pig and rams. But there is meager information on the immuno-castration of male goats (bucks). Black Bengal goats are well-known world-wide for its very good quality chevon. Keeping all these facts in view, the present study was designed to establish immuno-castration as a non-invasive and easy-to-do alternative to surgical castration in Black Bengal bucks that could also be of much interest to the people associated with the animal welfare movement.

MATERIALS AND METHODS

Experimental design

The experiment was conducted on thirty (30) healthy male Black Bengal bucks of 6-8 months of age. The goats were procured from a commercial farm. These goats weighed 10-12 kg each. All the animals were preconditioned to the animal house environment for two weeks to reduce stress and were closely observed in order to check up their health status before the beginning of the

experiment. Accordingly, necessary corrective measures like vaccination, deworming etc. were undertaken. The animals were randomly allocated to three groups, each containing ten bucks ($n = 10$). Group I bucks were used as control animals (C), Group II animals for surgical castration (SC) and the remaining Group III animals for immunological castration (IC). Control animals (Group I) were intact, non-castrated and non-immunized bucks. Surgical castration was routinely performed in Group II animals following usual anesthetic procedure and asepsis. All the bucks were maintained in individual pens and were fed the standard commercial diet with *ad-libitum* safe drinking water throughout the experimental period. The animal house was properly lighted and well ventilated. Before commencement of the experiment the necessary permission was obtained from the "Institutional Animal Ethical Committee", College of Veterinary Science and Animal Husbandry, Bhubaneswar. All the experimental procedures and protocols were duly approved by the committee.

Immunization procedure

Active immunization against GnRH was performed by injection of 2 ml of an emulsion containing equal volume of Freund's Complete Adjuvant (FCA) and saline containing 1 mg of GnRH-KLH (Keyhole limpet hemocyanin) conjugate subcutaneously in dorsal part of the upper neck region of the Group III animals. A booster subcutaneous injection of 2 ml was given 2 weeks after primary injection on the other side of the neck region of the said animals. The injectable solution was prepared as per the standard methodology (Adams and Adams 1990, 1992).

Determination of anti-GnRH antibody titers

Ten ml of blood samples were collected in heparinized tubes from each animal by jugular venipuncture on days 0, 1, 3, 5, 7, 15, 30, 45, 60, 85, 100, 115 till slaughter of the animals. Each blood sample was then centrifuged at 15000 rpm for 20 minutes. After that the plasma samples were aspirated and stored at -20°C . Anti-GnRH antibody titer was measured from the samples collected from Group III animals by using the procedure as described by Jago *et al.* (1997). It was expressed as the percentage (%) of total GnRH bound to antibody at 1:1000 dilution of serum.

Assays for plasma testosterone and plasma cortisol concentration

Plasma testosterone and cortisol level were measured for all blood samples collected from all the animals by

using ELISA reader (Merck, Japan) following the standard protocol (Srivastava 2002). The ELISA kit for testosterone measurement was supplied by IBL International, Hamburg, Germany and the cortisol kit was procured from National Institute of Health and Family Welfare, New Delhi, India.

Assessment of sexual behavior

Once before immuno-castration (on 10th day pretreatment) and twice thereafter (on day 45 and 100 days), the assessment of sexual behavior was done during 30 minutes test period placing each buck in a pen containing two estrous adult (female) goats on the basis of frequency of mounts, attempted mounts, ejaculation, head pushing, fighting, sniffing etc. At the same time, temperaments of the immuno-castrates were closely observed.

Assessment of semen quality

Semen evaluation was done after collection of ejaculated semen from artificial vagina in respect of ejaculate volume, density, motility, concentration etc. Sperm concentration was estimated by using hemocytometer. The semen quality assessment was performed in each of the bucks by following the procedure as described by Garner and Hafez (1986).

Statistical analysis

The means, standard error, standard deviation and coefficient of variation for variables under study were computed with the help of standard statistical procedure described by Snedecor *et al.* (1989). Duncan's Multiple Range Tests as modified by Kramer was used to test the difference among sub means. Data were analyzed by using IBM SPSS 23.0 statistical software.

RESULTS AND DISCUSSION

Reaction at injection site for immunization

A transient reaction at immunization site (in neck region) appeared to be only limited to the subcutaneous plaque formation in case of four animals, that subsided within a week. However, none of the animals showed any systemic reaction. Similar observation was reported by Godfrey *et al.* (1996).

Anti GnRH antibody titer

Antibody titers to GnRH were observed high 45 to 50 days after primary immunization in the respective group of bucks. The titer then increased to a much higher level towards the end (115 days) of the experimental period as shown in graphical representation, *i.e.* Fig. 1. The rise in titer of anti GnRH antibody in the present study followed the pattern similar to the earlier reports (Schanbacher 1982, Kiyma *et al.* 2000, Ulker *et al.* 2005) on ram lambs. Our observation is also consistent with those in cattle, goats and rodents, indicative of more potency of FCA than most other such adjuvant (Goubau *et al.* 1989, Bennet *et al.* 1993).

Plasma testosterone level

Plasma testosterone level did not differ among treatment groups at the beginning of the experimental period. Its concentration increased progressively throughout the experimental period in control group of animals. The surgically castrated bucks showed marked reduction in plasma testosterone level soon after the castration. The concentration of testosterone was also observed to be low in the bucks after immunization against GnRH. There was much reduction in plasma testosterone level about 45 days after primary immunization. Its concentration was reduced to an almost undetectable level

Table 1. Sexual behavioral traits (mean ± SE) in control, surgically castrated and immunized Black Bengal bucks (data from three assessment periods are combined).

Behavioural traits	Castrated bucks	Immunized bucks	Control bucks
Attempted mounts	0.9 ^a ±0.2	0.6 ^a ±0.5	3.1 ^b ±0.4
Mounts	0.5 ^a ±0.3	0.8 ^a ±0.4	6.5 ^b ±0.8
Ejaculations	0	0.2±0.2	4.1±0.6
Foreleg kicking	0.8 ^a ±0.5	0	2.2 ^b ±0.5
Nose to nose sniffing	1.2 ^a ±0.2	1.4 ^a ±0.4	3.2 ^b ±0.6
Udder sniffing	0.5 ^a ±0.1	0.8 ^a ±0.3	1.8 ^b ±0.3
Flehmen behaviour	0	0	0.3±0.2

*Means with different superscripts within a group showed significant difference, p<0.01.

Table 2. Semen characteristics of control (I) and immunized (III) Black Bengal bucks.

Days	Groups	Volume(ml)	Motility (%)	Sperm count (10 ⁹ /ml)	Density score
0	I	0.88±0.02	90.00±0.02	2.27±0.01	3.53±0.02
	III	0.90±0.02	58.49±0.50	2.26±0.03	3.55±0.02
15	I	0.92 ^a ±0.02	60.05 ^a ±0.13	2.30 ^a ±0.01	3.62 ^a ±0.02
	III	0.76 ^b ±0.03	58.90 ^b ±0.27	2.21 ^b ±0.01	3.62 ^a ±0.02
30	I	0.93 ^a ±0.02	59.97 ^a ±0.19	2.40 ^a ±0.04	3.59 ^a ±0.03
	III	0.70 ^b ±0.01	30.24 ^b ±0.64	0.03 ^b ±0.01	1.38 ^b ±0.09
60	I	0.95 ^a ±0.02	75.67 ^a ±0.30	2.47 ^a ±0.02	3.81 ^a ±0.03
	III	0.45 ^b ±0.02	10.18 ^b ±0.23	0.01 ^b ±0.00	1.16 ^b ±0.03
115	I	1.20 ^a ±0.04	78.27 ^a ±0.23	2.50 ^a ±0.01	3.77 ^a ±0.02
	III	0.31 ^b ±0.02	7.43 ^b ±0.64	0.01 ^b ±0.00	0.74 ^b ±0.08

*Means with different superscripts within a group showed significant difference, p<0.01.

in the immunized bucks towards the end of the experimental period, i.e. 100 days onward (Fig. 2).

The increasing antibody titer in immunized bucks about 45 days after primary immunization was in consonance with the decreasing plasma testosterone level observed during the same period. This indicates the fact that the production of high antibody titer against the neuropeptide GnRH was enough to suppress or neutralize the circulating level of endogenous GnRH secreted from hypothalamus that subsequently blocks pituitary gonadotropin secretion leading to the blockage of testosterone synthesis (Candek-Potokar *et al.* 2017). This sort of hypothesis (mechanism of action) and result were earlier reported by several researchers (Schanbacher 1982 in ram lambs, Adams and Adams 1992 in bulls). Similar effects of immunization in ram lambs were also observed by different workers (Kiyma *et al.* 2000, Cui *et al.* 2003, Ulker *et al.* 2005).

Plasma cortisol level

The immunization did not cause marked change in plasma cortisol level. Its concentration slightly increased post treatment for next few days and thereafter gradually decreased to almost normal level as compared to the control bucks. The initial post castration increase in plasma cortisol level in surgically castrated bucks was relatively much higher than in the immunized bucks (Fig. 3). Moreover, the level of physical stress in treated animals was manifested in their postures for the first few days post treatment. Thereafter, such behavioral changes were not noticed. Plasma cortisol level, an important stress indicator, was measured to ascertain the stress response of the immunized bucks and surgical castrates. Any type

of stress causes an increased secretion of cortisol in goat (Guyton and Hall 1996, Das 2000). Moreover, cortisol was considered as an important stress indicator by several workers (Cohen *et al.* 1990). Some postural changes (recumbency, restlessness, tail wagging etc.) observed in treated animals was due to physical stress inflicted on them as earlier reported by Robertson (1994). From the present study, it can be concluded that the immuno-castration technique was less stressful when compared with the surgical castration.

Assessment of sexual behavior

Surgically castrated bucks showed conspicuous reduction in several measures of sexual behavior like frequency of mounts, attempted mounts, ejaculation etc. when compared to the control group of bucks. Sexual behavior of immunized bucks was comparable to that of the castrated ones. All the available data are presented in Table 1. Both surgical and immuno-castrates were easily handled and managed than the untreated (control) animals. Sexual behavioral traits were affected by treatment. Both the treatment groups showed reduced libido and other sexual behaviors. Soon after the effective immunization, aggressive and mounting behavior was reduced, while feeding behavior became alike to surgical castrates (Rydhmer *et al.* 2010). Similar studies were conducted to find out the effect of GnRH neutralization in goats (Godfrey *et al.* 1996) and in bulls (Huxsoll *et al.* 1998). A reduced libido was reported in boars (Eshenshade and Johnson 1987) and in bulls (Jago *et al.* 1997). The observations of the present study are in conformity with the earlier reports in rams by Brown *et al.* (1994); Kiyma

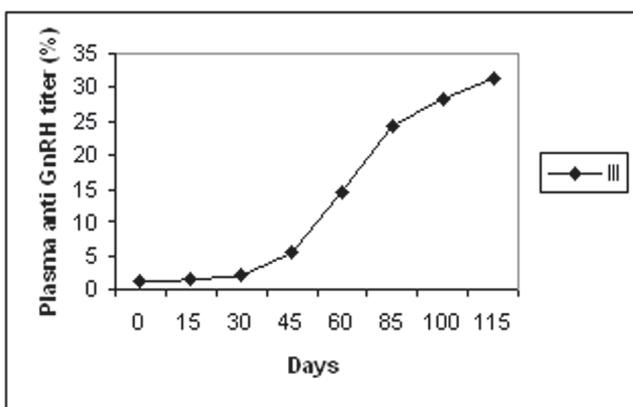


Fig. 1. Change in anti GnRH antibody titer (%) in immunized (Group III) Black Bengal bucks.

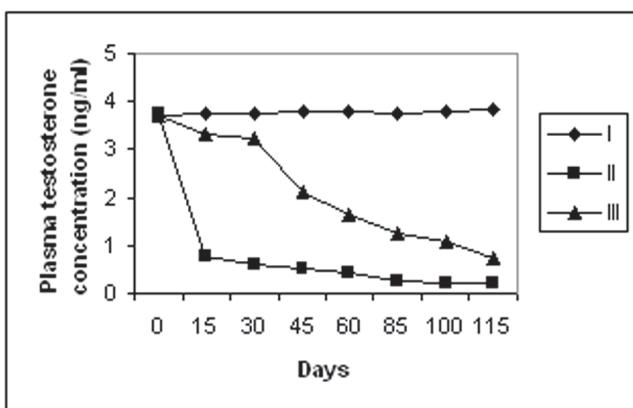


Fig. 2. Graphical representation of change in plasma testosterone level (ng/ml) in control (Group I), surgical castrates (Group II) and immunized (Group III) Black Bengal bucks.

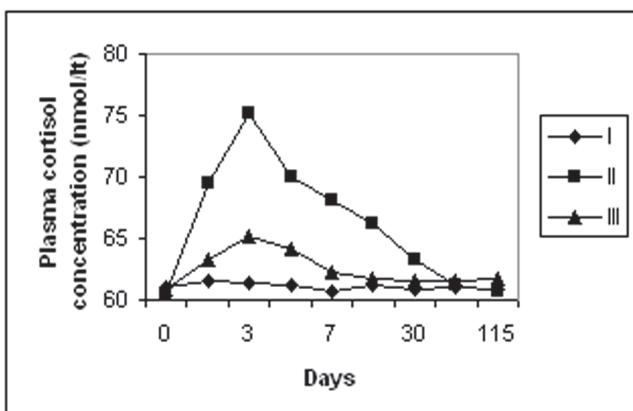


Fig. 3. Graphical representation of change in plasma cortisol level (nmol/L) in control (Group I), surgical castrates (Group II) and immunized (Group III) Black Bengal bucks.

et al. (2000), Cui *et al.* (2003) and Ulker *et al.* (2005) in ram lambs. The suppression of sexual behavior in immunized animals is due probably to the immunization

induced reduction in serum testosterone concentration. Accordingly, these animals became more docile and were easily managed during the experimental period.

Semen characteristics

Semen was collected by artificial vagina method from each and every buck on days 15, 30, 60, 115 and was assessed in respect of volume, sperm concentration, motility and density. Semen collected from the immunized bucks showed only a few live spermatozoa and even almost azoospermia towards the end of the experiment (Table 2). Besides, the motility, density and volume of ejaculate from immunized bucks were significantly lower as compared to the control group ($p < 0.001$). It is evident from Table 2 that the semen quality gradually deteriorated in the immunized bucks. Our findings in this regard were comparable with the earlier reports (Godfrey *et al.* 1996 in goat bucks, Brown *et al.* 1994 in rams). Ulker *et al.* (2005) did not observe the immunized ram lambs to produce mature sperms. Significantly reduced sperm concentration was recorded by different workers (Adams and Adams 1992 in bulls immunized against GnRH, Turkstra *et al.* 2005 in stallions). Some study elucidated almost same result on the semen quality of immunologically castrated bulls (Robertson *et al.* 1979, 1982). Ejaculates from immunized boars were devoid of cellular components (Esbenshade and Johnson 1987). Marked reduction in percentage of live sperm and even azoospermia as well as some other qualitative parameters of semen in immunized bucks is the result of suppressed gonadal (testicular) development and function due to much lower concentration of plasma testosterone as mentioned earlier.

Immuno-castration uses the natural immune system of the animal to achieve the effects of castration. The production of high antibody titer against the neuropeptide GnRH, neutralizes the endogenous GnRH secreted from hypothalamus and blocks the stimulatory action of this hormone on pituitary gonadotropin secretion (*i.e.* blocks hypothalamic – pituitary – gonadal axis). This event not only blocks consequently the gonadal steroids (testosterone) synthesis, but in turn, suppresses the testicular development and spermatogenesis also (Candek-Potokar *et al.* 2017). Though the optimum period needed between primary and booster immunizations has not been fully addressed in different species, a 2-week interval between 2 immunizations in the present study produced effective results. It's most likely that a yearly booster may be sufficient to maintain immuno-castration effects in goats (Godfrey *et al.* 1996). A single injection

protocol may be more effective at or before onset of puberty in these animals and its effects last till their entry to market (7-8 months) as proposed by Cui *et al.* (2003).

CONCLUSION

Active immunization against GnRH of prepubertal and pubertal bucks attains most of the characteristic features similar to the surgical castrates. Therefore, immuno-castration may be a non-invasive, appropriate alternative to conventional castration techniques, the former being more convenient and acceptable for use and having no significant or potential side effects.

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